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Rat pineal Gsα, Giα and Goα: relative abundance and development

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The adult rat pineal gland contains relatively high concentrations of Gsα, low amounts of both Giα and Goα, and undetectable levels of Gtα. During development the amounts of 45 kDa Gsα and of Giα remain constant. In contrast, 42 kDa Gsα and Goα are nearly absent at birth and increase in abundance markedly thereafter. Gtα is undetectable at any age. It would appear that multiple mechanisms regulate the expression of G-proteins in the pineal gland.

The rat pineal gland is a popular model of signal transduction which has provided new information about how second messengers are regulated[9]. However, little is known about GTP-binding regulatory proteins in the pineal gland, except for a significant amount of indirect evidence indicating that the α subunit of the stimulatory GTP-binding protein (Gsα) is present in the tissue[3–5,13,15,19,22,24] and unpublished evidence indicating that a pertussis toxin substrate, presumably the α subunit of the inhibitory GTP-binding protein (Giα), is also present[19]. In the present report we have determined the relative abundance and development of pineal Gsα, Giα, Goα (Go stands for other, unknown function), and the α subunit of transducin (Gtα).

Membranes were obtained by sonication in a buffer containing: 0.5 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 21 μM leupeptin, 20 mM Tris-HCl, pH 7.4. The homogenate was then centrifuged (4°C, 1 h, 100,000 *g*). The resulting pellet was washed and resuspended in the sonication buffer. Protein was estimated using a dye-binding method[2].

Membrane proteins were resolved by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis[12], and transferred from gels onto PVDF membranes (Immobilon P^{TM}, Millipore Corp., Bedford, MA)[20]. Electroblots were probed with either EK/2 (anti-Gtα_{2-17}) for detection of Gtα (William F. Simonds, personal communication); RM (anti-Gs$\alpha_{385-394}$)[16] for detection of Gsα; AS/7 (anti-Gt$\alpha_{341-350}$)[7] for detection of Giα_1 and Giα_2; or GO (anti-Go$\alpha_{345-354}$)[6] for detection of Goα. The immunoreaction was visualized by an autoradiographic method using ^{125}I-protein A (100,000 cpm/ml 0.05% Tween in Tris-buffered saline). Autoradiograms were visualized by a CCD camera (Sierra Scientific) above a light box of variable intensity (Illuminator model 890, Imaging Research Inc.). The density of immunopositive bands was measured with the IMAGE program running on a Mackintosh II[14]. Membrane associated Gsα, Giα, and Goα in 14 tissues were analyzed; Gtα was studied in the pineal gland and retina.

Gsα. Gsα (42 and 45 kDa) in the pineal gland was relatively high (Fig. 1): pineal ~cerebral cortex ~cerebellum > hypothalamus ~retina ~adrenal > thyroid ~ovary > kidney ~spleen ~liver ~testes > heart ~lung. The presence of Gsα in the pineal is consistent with the results of previous reports, as described above. The presence of two species of Gsα is typical of this protein, and is consistent with unpublished evidence that the pineal gland contains two substrates of cholera toxin[18]. The high concentration of Gsα in the pineal gland as compared to other tissues may explain the robust adrenergic stimulation of cyclic AMP in the pineal gland[9].

Analysis of the developmental appearance of Gsα (Fig. 2) revealed that there was a marked difference in the development of the two forms of Gsα (M_r 42 and 45 kDa). The 45 kDa protein was present in the fetal and adult rat at nearly identical levels, with a significant increase at day 3 ($P < 0.05$). In contrast, the 42 kDa form of Gsα was relatively low at birth and increased markedly (7-fold) between day 7 and day 40. Assuming that both forms of Gsα are equally immunoreactive, the total Gsα immunoreactivity was relatively constant throughout development. However, the ratio of the 45 kDa:42 kDa form decreases from about 11 in the new-

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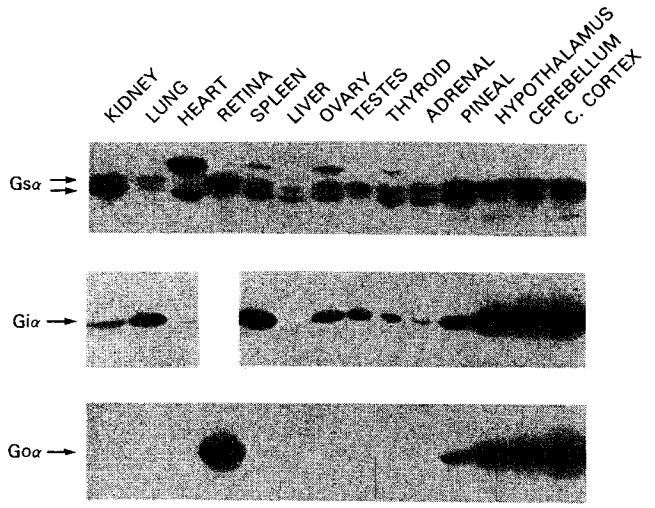

Fig. 1. Tissue distribution of Gsα, Giα and Goα. Gsα, Giα and Goα immunoreactivity in selected adult rat (60 days old) tissues. A 100 μg sample of membrane protein was loaded per lane of a 12.5% polyacrylamide gel. Proteins were electroeluted onto PVDF membranes and immunodetected (dilutions are given in parentheses) with anti-$Gs\alpha_{385-394}$ serum (RM; 1:500), anti-$G\tau\alpha_{341-350}$ serum (AS/7; 1:250, this antiserum reacts with $Gi\alpha_1$ and $Gi\alpha_2$), and anti-$Go\alpha_{345-354}$ serum (GO; 1:250). For further details see the text.

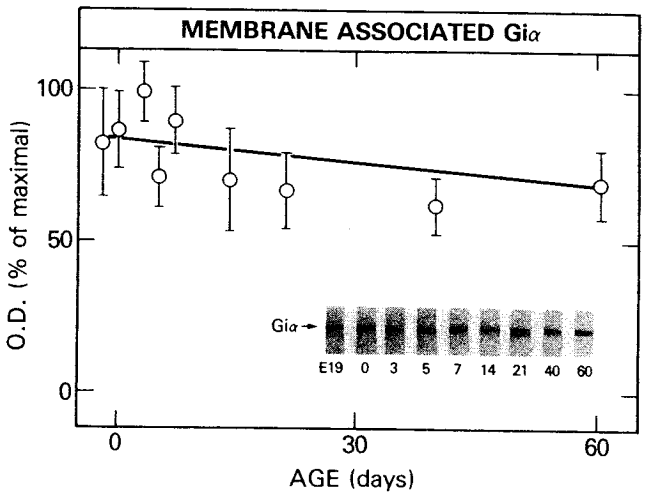

Fig. 3. Development of pineal membrane Giα. Pineal membrane preparations (50 μg) from rats of the indicated ages were analyzed. Giα was detected using a rabbit anti-$G\tau\alpha_{341-350}$ serum (AS/7; 1:250) which reacts with $Gi\alpha_1$ and $Gi\alpha_2$. Normalized data are presented. Quantitation was performed according to O'Neill et al.[14]. Photographs of typical immunoreactive bands are presented in the insert. Data are based on material prepared from 4 collections of tissue; each time point represents the mean ± S.E.M. of the % of maximum O.D. For further details see the legend to Fig. 1 and the text.

born to about 2 in the adult (insert in Fig. 2).

The presence of the large form of Gsα at birth suggests that it alone might mediate adrenergic or cholera toxin stimulation of cAMP, which is easily demonstrated early in life[1]. In contrast, the later appearance of the small form of Gsα is roughly similar to developmental appearance of the cGMP response to adrenergic stimulation (unpublished data) and to the appearance of hydroxyindole-*O*-methyltransferase activity[10,17]. This raises the possibility that there might be a functional relation-

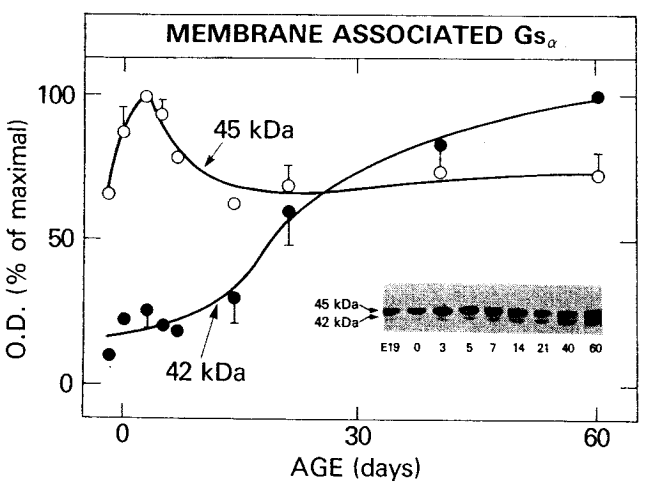

Fig. 2. Development of pineal membrane Gsα. Pineal membrane preparations (50 μg) from rats of the indicated ages were analyzed. Gsα was detected using a rabbit anti-$Gs\alpha_{385-394}$ serum (RM; 1:500). Normalized data are presented. Quantitation was performed according to O'Neill et al.[14]. Photographs of typical immunoreactive bands are presented in the insert. Data are based on material prepared from 4 collections of tissue; each time point represents the mean ± S.E.M. of the % of maximum O.D. The absence of an error bar indicates the S.E.M. fell within the symbol. For further details see the legend to Fig. 1 and the text.

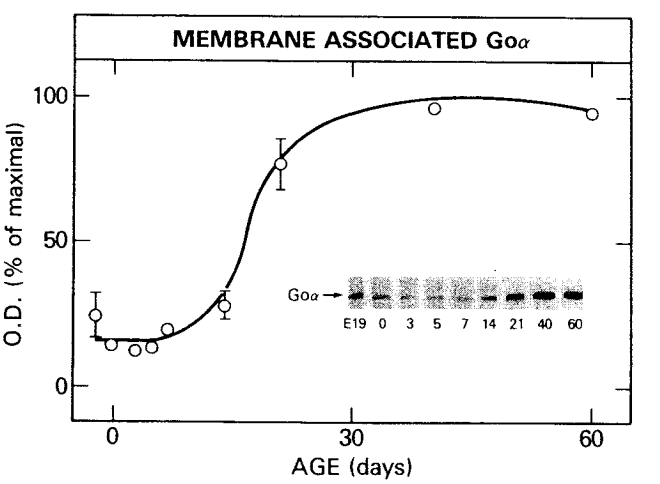

Fig. 4. Development of pineal membrane Goα. Pineal membrane preparations (50 μg) from rats of the indicated ages were analyzed. Goα was detected using a rabbit anti-$Go\alpha_{345-354}$ serum (GO; 1:250). Normalized data are presented. Quantitation were performed according to O'Neill et al.[14]. Photographs of typical immunoreactive bands are presented in the insert. Data are based on material prepared from 4 collections of tissue; each time point represents the mean ± S.E.M. of the % of maximum O.D. The absence of an error bar indicates that the S.E.M. fell within the symbol. For further details see the legend to Fig. 1 and the text.

ship between the smaller form of Gsα and either the cGMP response or hydroxyindole-*O*-methyltransferase activity.

The existence of multiple forms of Gsα is known to reflect variations in RNA splicing. Thus, it would appear that the mechanisms required for generation of mRNA encoding the small form of Gsα might not operate in the pineal gland early in life. For example, an essential enzyme may not be present, or such an enzyme might be present in an inactive form. Alternatively, differences in the degradation of message or of protein or both might explain the observed differences in the developmental patterns of the two forms of Gsα.

Giα. Giα was studied using an antiserum (AS/7) which detects $Gi\alpha_1$ and $Gi\alpha_2$ with much greater reactivity than $Gi\alpha_3$[8]. Accordingly, positive Giα signals presented here probably reflect the presence of $Gi\alpha_1$ or $Gi\alpha_2$ or both, but not the likely presence of $Gi\alpha_3$. Using AS/7 it appeared that the abundance of $Gi\alpha_1$ and $Gi\alpha_2$ in the pineal gland was relatively low (Fig. 1): cerebral cortex > cerebellum > hypothalamus > pineal ~kidney ~spleen ~adrenal ~heart ~testes ~ovary ~thyroid ~liver. In other studies we found that two molecular species of Giα, probably $Gi\alpha_1$ and $Gi\alpha_2$, were detected inconsistently in the pineal gland, perhaps reflecting gel-to-gel differences in resolving efficacy. Results from the retina are not presented because they are ambiguous: the strong immunoreaction produced by AS/7 with retinal extracts is probably primarily due to Gtα, which is abundant in this tissue, and to an unknown degree to reaction with Giα. As indicated above, AS/7 was originally raised against Gtα, but also reacts with $Gi\alpha_1$ and $Gi\alpha_2$.

Giα was present at all stages of development at constant levels (Fig. 3). This establishes that the potential exists for Giα to negatively regulate adenylyl cyclase activity throughout life in the pineal gland. The possibility that Giα plays a role in pineal signal transduction has been considered[19]. One hypothesis proposes that α_1-adrenergic activation of protein kinase C could cancel inhibitory influences of Gi and allow full Gsα stimulation of adenylyl cyclase.

Goα. The pineal gland was found to contain a relatively low amount of Goα (Fig. 1): cerebral cortex > cerebellum > hypothalamus ~retina > pineal. Goα was undetectable in other tissues. Two species of Goα were detected in the pineal gland on an inconsistent basis. In contrast to Giα, the developmental pattern of Goα was similar to that of the 42 kDa form of Gsα, with O.D. values exhibiting a 5-fold increase between day 7 and day 40 (Fig. 4).

Gtα. Gtα was undetectable during all stages of development in the pineal gland (in these experiments retinal Gtα was unambiguously detected, data not presented), in agreement with previous observations made on adult mammals[21]. It is known that Gtα is present in the pineal gland of lower vertebrates, where it functions together with rhodopsin to mediate phototransduction[21]. Opsin is present in the neonatal rat pineal gland[11] (Babila and Klein, unpublished data), raising the possibility that this tissue might be photosensitive. However, the absence of Gtα at this time makes it seem highly improbable that the neonatal rat pineal gland is capable of phototransduction.

Day–night analysis of pineal Gsα, Giα and Goα. Preparations were obtained from animals killed at 4 times of the day (05.00 h, 11.00 h, 19.00 h and 24.00 h). These animals had been housed for two weeks in a 14:10 lighting cycle (lights on at 07.00 h). Western blot analysis, as described above, did not reveal a significant difference in the level of Gsα, Giα or Goα at any time point (data not presented). The absence of such changes indicates that these proteins do not fluctuate markedly in response to the normal day/night difference in neural stimulation of the pineal gland.

In summary, it is interesting to note that the developmental profiles of the individual α subunits of G-proteins in the pineal gland exhibit two distinctly different patterns. One, which describes the development of the large species of Gsα and Giα, is characterized by high levels throughout life. Obviously this reflects the early expression of genes, and may be functionally associated primarily with cyclic AMP production. It seems reasonable to suspect that the ontogenetic expression of both these G-proteins may reflect a common switch. Another set of controls seems to determine the coordinated developmental appearance of the small form of Gsα and of Goα. A common mechanism might trigger the appearance of both these proteins. The nature of this switch is of interest. It might reflect a predetermined developmental schedule or an extrapineal signal. One such extra pineal signal might be sympathetic stimulation, which starts during the period in which these proteins are first expressed[23].

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